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Intimin subtyping of atypical enteropathogenic *Escherichia coli* isolated from children with and without diarrhea: a possible temporal shift in the distribution of intimin alleles☆,☆☆

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ABSTRACT

Intimins of atypical EPEC strains from children with and without diarrhea were genotyped. κ was not found and β was the most common. η- and ζ-alleles prevailed in strains from children without diarrhea and ι-allele among children older than 13 months. ε-allele emerged in 2006 and was the most common in 2007.

Enteropathogenic *Escherichia coli* (EPEC) is a leading cause of diarrhea in less economically favored regions (Chen and Frankel, 2005). EPEC strains are defined as intimin-containing diarrheagenic *E. coli* (DEC) that produce an attaching-and-effacing (A/E) lesion on enterocytes but do not express Shiga toxin (Garmendia et al., 2005; Kaper, 1996; Nataro and Kaper, 1998). These strains are classified as typical EPEC (t-EPEC) when they harbor *bfp* on a plasmid named EAF or as atypical EPEC (a-EPEC) which do not carry EAF (Trabulsi et al., 2002). t-EPEC strains are isolated only from human feces, whereas a-EPEC strains have been found in different animals (Blanco et al., 2005; Cortés et al., 2005; Orden et al., 2003; Trabulsi et al., 2002).

Intimin, which binds the translocated intimin receptor, is an outer-membrane adhesin that is essential for colonization of the intestine by A/E pathogens including EPEC (Mundy et al., 2007). The N-terminus of intimin is highly conserved, while the C-terminus, where the active receptor-binding site resides, shows sequence polymorphism (Frankel et al., 1995). This diversity defines distinct intimin types, the most common being α, β, and γ, depending on the A/E *E. coli* pathotype and source and geographical location (Adu-Bobie et al., 1998; Mundy et al., 2007). Intimin α is associated with EPEC, intimin γ is common in EHEC, while intimin β appears to be the most ubiquitous being found among human and animal A/E pathogens. Furthermore, there are several evidences that suggest that intimin alleles influence host specificity and tissue tropism (Girard et al., 2005; Phillips and Frankel, 2000).

Taking into account the scarcity of studies on the distribution of intimins, we undertook this investigation in order to characterize the a-EPEC strains isolated during a study of infectious diarrhea in Belo Horizonte, Brazil, by intimin genotyping. The association between the results obtained and clinical, demographic, and epidemiologic data was evaluated.

This study is part of a project aiming to understand better the pathogenesis of infectious diarrhea in children with and without diarrhea. It was approved by the ethical committee of the Universidade Federal de Minas Gerais. Feces were obtained from 790 children (49.7% with diarrhea, 55.9% boys, 54.6% in drier months) aged up to 5 years, from 2004 to 2007, in Belo Horizonte, Brazil. *E. coli* was isolated by conventional methods and a-EPEC was identified by a polymerase chain reaction (PCR) protocol (Vidal et al, 2005). Intimin subtyping was performed by PCR according to a method which identifies 9 intimin subtypes (Zhang et al, 2002). Clinical, demographic, and epidemiologic data were obtained from each patient and stored in a databank (SPSS Statistics, version 19.0.0, SPSS, Chicago, IL, USA). The χ^2 test with Yates' correction or Fisher's exact test was used for comparisons between groups, as appropriate. Differences were taken as significant when the P value was <0.05.

a-EPEC was isolated from 90 (11.9%) subjects. Among them, 4 (4.4%) were co-infected with t-EPEC and 2 (2.2%) with EHEC, findings

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Table 1
Association between diarrhea, sex, and seasonality, and intimin genotypes detected in atypical EPEC isolated from 73 children with and without diarrhea.

Intimin[a]	Diarrhea		Sex		Season	
	With	Without	Male	Female	Rainy	Dry
α	5 (15.6)[b]	3 (7.3)	5 (12.2)	3 (9.4)	4 (11.8)	4 (10.3)
β	12 (37.5)	15 (36.6)	15 (36.6)	12 (37.5)	13 (38.2)	14 (35.9)
ε	3 (9.4)	4 (9.8)	3 (7.3)	4 (12.5)	1 (2.9)	6 (15.4)
η	1 (3.1)	7 (17.1)	6 (14.6)	2 (6.3)	4 (11.8)	4 (10.3)
γ	4 (12.5)	3 (7.3)	3 (7.3)	4 (12.5)	3 (8.8)	4 (10.3)
ι	3 (9.4)	1 (2.4)	4 (9.8)	0 (0.0)	3 (8.8)	1 (2.6)
θ	2 (6.3)	0 (0.0)	1 (2.4)	1 (3.1)	1 (2.9)	1 (2.6)
ζ	0 (0.0)	5 (12.2)	1 (2.4)	4 (12.5)	3 (8.8)	2 (5.1)
Mixed	2 (6.3)	3 (7.3)	3 (7.3)	2 (6.3)	2 (2.9)	3 (7.7)
Total	32 (100)	41 (100)	41 (100)	32 (100)	34 (100)	39 (100)

[a] Intimin κ was not detected.
[b] Values are shown as *n* (%).

Table 3
Temporal distribution of intimin genotypes detected in atypical EPEC isolated from feces of children with and without diarrhea.

Intimin[a]	Year of the study				Total
	2004	2005	2006	2007	
α	1[b] (10.0[c]/12.5[d])	5 (16.7/62.5)	2 (10.0/25,0)	0 (0.0/0.0)	8
β	5 (50.0/18.5)	13 (43.3/48.1)	7 (35.0/25.9)	2 (15.4/7.4)	27
ε[e]	0 (0.0/0.0)	0 (0.0/0.0)	4 (20.0/57.1)	3 (23.1/42.9)	7
η	1 (10.0/12,5)	3 (10.0/37.5)	2 (10.0/25.0)	2 (15.4/25.0)	8
γ	0 (0.0/0.0)	3 (10.0/42.9)	2 (10.0/28.6)	2 (15.4/28.6)	7
ι	1 (10.0/25.0)	0 (0.0/0.0)	1 (5.0/25.0)	2 (15.4/50.0)	4
θ	1 (10.0/50.0)	1 (3.3/50.0)	0 (0.0/0.0)	0 (0.0/0.0)	2
ζ	0 (0.0/0.0)	3 (10.0/60.0)	0 (0.0/0.0)	2 (15.4/40.0)	5
Mixed	1 (10.0/20.0)	2 (6.7/40.0)	2 (10.0/40.0)	0 (0.0/0.0)	5
Total	10	30	20	13	73

[a] Intimin κ was not identified.
[b] Values are shown as *n* (%).
[c] Percentage calculated considering the total of intimin genotypes identified within each year.
[d] Percentage calculated considering the total of each intimin genotype identified in the study.
[e] $P = 0.025$.

that demonstrate the high frequency of mixed infection in our population. a-EPEC strains isolated from 84 (93.3%) children with nonmixed infection were further analyzed.

An intimin genotype was not identified in strains obtained from 11 (13.1%) children who were excluded from the analyses. The distribution of intimins among diarrhea and control patients and according to sex, season, and age, and along the period of the study is shown in Tables 1–3. Five children (6.8%) were colonized by strains harboring more than 1 intimin genotype: 2 patients by types β and η, and 1 each by α and γ, α and η, and γ and θ. These findings demonstrate further the high prevalence of mixed infection in our population.

We did not find intimin κ. Although this intimin allele is more common in animal feces, it is associated with more severe episodes of diarrhea in humans (Contreras et al, 2010). Similarly to findings reported for children from Germany and Australia (Beutin et al., 2003), β-intimin was the most common in our population. On the other hand, higher frequency of θ and γ alleles was reported for Uruguayan children with diarrhea and Danish children, respectively (Blanco et al, 2006; Jensen et al, 2007). Although no association has been reached, intimins α, γ, ι, and θ were more common in diarrhea patients (Table 1). A statistical trend towards an association between η- and ζ-intimin alleles and absence of diarrhea was found ($P = 0.069$ and $P = 0.062$, respectively). Similarly, higher prevalence of intimin θ in diarrhea patients and ζ in controls has been reported (Jensen et al, 2007). In contrast, Contreras et al. (2010) found an even distribution of intimins among children aged less than 12 months with and without diarrhea in Peru.

No association with sex was detected, but η and ι alleles were more frequent in males and ε, γ, and ζ in females. Similarly, the intimin alleles investigated were evenly distributed in rainier and drier seasons, but ι was more common in the rainier and ε in the drier season (Table 1). No data on seasonality and on sex distribution of intimins were found in the available literature.

When we stratified the intimins according to age (Table 2), β-intimin allele predominated in every age range, except for η in children older than 2 years. Genotype θ was found only in children less than 6 months of age, but β strains were the most common within this age range. Despite these observations, statistical association was reached only for ι-intimin ($P< 0.001$; Table 2) which was found exclusively in children older than 1 year. Contrarily to our findings, homogeneous distribution of the intimins was reported for Peruvian children aged less than 12 months (Contreras et al., 2010).

When data were stratified according to the year of the investigation, β-intimin was the dominant genotype across the period of the study except for the year 2007 when ε-allele prevailed. Also, the frequency of α-, β-, and γ-intimin alleles decreased along the period of the study, while ε-intimin emerged and became the predominant type in 2007 ($P = 0.025$). We are not aware of any report on this aspect of the infection by a-EPEC and we have no ready explanation for this finding. To our knowledge, there are insufficient data available on intimin alleles of a-EPEC circulating in humans around the world. Our findings could reflect the dynamics of a-EPEC circulation and could be explained by shifts originated under selective pressure of neutralizing antibodies. Although this thesis remains speculative, this is the first report on this aspect of a-EPEC infection in humans. Our data contribute for understanding better the worldwide distribution

Table 2
Age distribution of intimin genotypes identified in atypical EPEC strains obtained from children with and without diarrhea.

Intimin[a]	Age (months)					Total
	Up to 6	7 to 12	13 to 18	19 to 24	25 to 48	
α	2[b] (7.4[c]/25.0[d])	4 (18.2/50.0)	0 (0.0/0.0)	1 (16.7/12.5)	1 (16.7/12.5)	8
β	12 (44.4/44.4)	8 (36.4/29.6)	4 (33.3/14.8)	2 (33.3/7.4)	1 (16.7/3.7)	27
ε	5 (18.5/71.4)	2 (9.1/28.6)	0 (0.0/0.0)	0 (0.0/0.0)	0 (0.0/0.0)	7
η	1 (3.7/12.5)	3 (13.6/37.5)	1 (8.3/12.5)	1 (16.7/12.5)	2 (33.3/25.0)	8
γ	2 (7.4/28.6)	1 (4.8/14.3)	3 (25.0/42.9)	1 (16.7/14.3)	0 (0.0/0.0)	7
ι[e]	0 (0.0/0.0)	0 (0.0/0.0)	2 (16.7/50.0)	1 (16.7/25.0)	1 (16.7/25.0)	4
θ	2 (7.4/100)	0 (0.0/0.0)	0 (0,0/0.0)	0 (0.0/0.0)	0 (0.0/0.0)	2
ζ	1 (3.7/20.0)	3 (13.6/60.0)	0 (0.0/0.0)	0 (0.0/0.0)	1 (16.7/20.0)	5
Mixed	2 (7.4/40.0)	1 (4.8/20.0)	2 (16.7/40.0)	0 (0.0/0.0)	0 (0.0/0.0)	5
Total	27	22	12	6	6	73

[a] Intimin κ was not identified.
[b] Values are shown as *n* (%).
[c] Percentage calculated considering the total of intimin genotypes within each age group.
[d] Percentage calculated considering the total of each intimin genotype identified in the study.
[e] $P < 0.001$.

of the organism and may be useful for designing strategies for preventing a-EPEC infection.

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